

# Grower Summary

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**CP 141**

**The molecular basis of pathogenicity of  
*Neonectria ditissima***

Grower Summary 2018

**Project title:** The molecular basis of pathogenicity of *Neonectria ditissima*.

**Project number:** CP 141

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**Report:** Annual report, second year.

**Previous report:** Annual report, first year.

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**Date project commenced:** Oct 2015

**Date project completed** Oct 2018  
**(or expected completion date):**

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# GROWER SUMMARY

## Headline

- This project aims to provide fundamental insights into the molecular basis of pathogenicity in *Neonectria ditissima*, the causative agent of apple canker.

## Background and expected deliverables

European canker, caused by the phytopathogenic fungus *Neonectria ditissima*, is one of the most destructive diseases of apple and pear. In the orchard, this fungus is able to infect a wide range of apple varieties causing canker and die back of young shoots, resulting in significant losses of fruiting wood. This pathogen has been reported in many apple-producing regions of the world, being especially common in the North-Western European countries. Modern varieties suffer most and in extreme cases do not survive establishment in the orchard. Canker control is difficult to achieve due to the pathogen's lifestyle which is able to infect trees all year-round through wounds, either natural such as bud-scale scars, leaf scars and fruit scars or artificial such as pruning wounds.

Resistance breeding is underway in many global breeding programmes, but nevertheless, a total resistance to canker has not yet been demonstrated in either fruit or woody tissue. There is no known race structure of the pathogen and the global level of genetic diversity of the pathogen population is unknown. Plant resistance is a promising alternative to largely ineffective cultural control, but is time consuming to deploy due the long breeding cycle in apple.

Research into other host pathogen interactions shows that a dual strategy of understanding host resistance and pathogen avirulence and how the two are linked is key to the deployment of durable resistance in the field. Nevertheless, little is known about the pathogen at the molecular level.

This project is focused on dissecting components of the pathogen's genome that modulate virulence, in order to understand how virulence is controlled and whether there are specific differences in host resistance response to isolates of differing virulence. The identification of candidate genes important in virulence in the pathogen could lead to novel opportunities for control by targeted disruption of the pathogen.

## Summary of the project and main conclusions

It is widely known that cultivars vary in their susceptibility to canker, though the exact molecular mechanism is unknown. Pathogen variability and host responses can be assessed through screening tests *in planta*, to identify the optimal conditions for disease development. Host responses to different inoculum, different infection methods and at different physiological conditions are being compared in this project. The previous report presented the results of some pathology tests based on the assessments of host responses and discussed the inconsistencies observed in test performance compared with field observations. In this report, the differences in the pathogenicity of several isolates of *N. ditissima* are compared.

During the infection process, the pathogen secretes proteins, called effectors, to modulate the host cells' response suppressing defence and allowing the colonisation. The genome of *N. ditissima* facilitates the identification of putative effectors and pathogenicity genes through bioinformatics analysis of the secretome and through comparisons to other pathogens. In this report, the scientists annotated a previously sequenced *N. ditissima* genome assembly using RNA-Seq data and have now improved this assembly using PacBio long-reads sequencing, allowing them to present an updated analysis of the predicted secretome of this pathogen. This analysis showed a full repertoire of pathogenicity genes, composed of secreted effector proteins and enzymes involved in the cell degradation wall, distributed throughout the genome. Differences in isolates' pathogenicity might be associated with their gene content. Therefore, analysis and comparison of the genome sequences of isolates differing in virulence might aid the identification of specific regions in the genome related with the virulence. On the other hand, a gene expression analysis will be carried out during the time course of an infection sequencing RNA from infected host tissue. This will allow the identification of the specific genes controlling host defences and pathogen virulence. The identification of specific candidate genes controlling pathogen virulence will allow a better understanding of the mechanism of infection.

Once a comprehensive list of genes important to pathogenicity have been identified from genomic and transcriptomic comparisons, validation of gene function will be attempted by knockout of key gene targets in the pathogen through homologous recombination-mediated gene deletion and targeted transformation.

Identifying the nature of resistance to *N. ditissima* has been challenging in the past. Previous tests in apple seedlings from different crosses revealed that resistance responses to *N.*

*ditissima* are complex and involved multiple resistance genes, with epistatic interaction modulating inheritance. Phenotyping resistance responses of an offspring from a single bi-parental population, 'Golden Delicious' x 'M9', through previously defined screening tests, will be useful to explore genetic variation in resistance. With this data, the scientific team will be able to carry out a QTL mapping associated with the resistance to *N. ditissima*.

### **Financial benefits**

- This research project is unlikely to offer any immediate financial benefits to growers, but may lead to the development of novel opportunities for the long-term control of apple canker.

### **Action points for growers**

- No direct action points for growers are likely to be delivered by this project but the results will be used in developing novel methods of management and control of apple canker in future.